

7 CARBON MONOXIDE CONFIRMATION BY PALLADIUM CHLORIDE MICRODIFFUSION	Page 1 of 2
<div> Division of Forensic Science TOXICOLOGY TECHNICAL PROCEDURES MANUAL </div>	Amendment Designator:
	Effective Date: 31-March-2004
<div> 7 CARBON MONOXIDE CONFIRMATION BY PALLADIUM CHLORIDE MICRODIFFUSION 7.1 Summary 7.1.1 Carbon monoxide is a volatile gas with strong reducing properties. Carbon monoxide is liberated from blood by strong acid in a microdiffusion cell and palladium chloride, in the center of the diffusion cell, is reduced to metallic palladium which has a silver appearance. The presence of CO in blood can thus be easily detected by observation of the appearance of the silver film. 7.2 Specimen Requirements 7.2.1 Approximately 2 mL of blood or mixed tissue containing sufficient quantity of hemoglobin 7.3 Reagents and Standards 7.3.1 Hydrochloric Acid, concentrated 7.3.2 Sulfuric Acid, concentrated 7.3.3 Palladium Chloride 7.3.4 0.1 N Hydrochloric Acid: Cautiously add 8.3 mL of concentrated HCl to approximately 100mL of dH₂O in a 1 L volumetric flask. QS to volume with dH₂O. 7.3.5 10% (3.6 N) Sulfuric Acid: Cautiously add 10 mL of concentrated H₂SO₄ to approximately 70 mL of dH₂O in 100 mL volumetric flask. Cool and QS to volume with dH₂O. 7.3.6 0.005 N Palladium Chloride Reagent: Weigh 0.22 g palladium chloride and transfer into a in 250 mL volumetric flask. QS to volume with 0.1 N HCl and let stand overnight. Transfer to a 500 mL volumetric flask and QS to volume with 0.1 N HCl. 7.4 Calibrators, Controls, and Internal Standards 7.4.1 IL TestTM Multi-4TM CO-Oximeter Controls, Instrumentation Laboratory Company, Lexington, MA. 7.5 Apparatus 7.5.1 Conway microdiffusion cells (2 well, with cover) 7.5.2 Sealant or dH₂O 7.6 Procedure 7.6.1 Prepare microdiffusion cell with sealant or dH₂O. 7.6.2 Add 2 ml of PdCl₂ reagent to center well of microdiffusion cell 7.6.3 Add 2 ml blood to one side of the outer ring 7.6.4 Add 1 ml of 10% H₂SO₄ to the other side of the outer ring. Quickly cover microdiffusion cell and gently rock/rotate to mix blood with sulfuric acid. Diffuse for approximately one hour. 7.6.5 Record results </div>	

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<div> 7.7 Quality Control and Reporting </div> <div> 7.7.1 A silver colored mirror will form in the center well of the dish in positive samples. Negative samples will appear to be unchanged (clear yellow gold color of the palladium chloride reagent). The intensity of the silver mirror will be directly proportional to the concentration of carbon monoxide in the blood. </div> <div> 7.7.2 A very small but noticeable silver mirror (particles) indicates about 10% saturation which is the LOD. </div> <div> 7.7.3 Record the intensity of the reaction by using “+++” to indicate the strongest reaction (e.g. >60% saturation), ++ (e.g. 30-50% saturation), and + (e.g. 10-20% saturation) </div> <div> 7.7.4 Analyze at least one level of the positive controls and a negative control with each group of case samples. </div> <div> 7.8 Notes </div> <div> 7.8.1 Sulfur compounds (e.g. hydrogen sulfide from putrified specimens) may react with PdCl₂. For putrified specimens, substitute lead acetate for 10% sulfuric acid and allow to diffuse for 4 hours. </div> <div> 7.9 References </div> <div> 7.9.1 Williams, L.A., <u>Methodology for Analytical Toxicology</u> , CRC Press, I. Sunshine, editor, Cleveland, 1975. </div>	